

# **Role of the Small Integrin Binding Ligand N-linked Glycoprotein (SIBLING), bone sialoprotein (BSP) in bone development and remodeling.**

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## **Between cell and mineral - the SIBLINGs**

The « small, integrin binding ligand, N-linked glycoprotein » family (SIBLINGs, [1]) group osteopontin (OPN), bone sialoprotein (BSP), dentin sialophosphoprotein (DSPP), dentin matrix protein-1 (DMP-1) and matrix extracellular glycohosphoprotein (MEPE). The genes for this family are aligned on a portion of human chromosome 4 (mouse chromosome 5), within a « Bone Gene Cluster » [2, 3] grouping other genes of bone interest. Molecular evolution studies [4, 5] suggest that SIBLINGs, along with enamelines and other proteins found in milk (caseins) and saliva (statherin), form a « Secretory Calcium binding PhosphoProteins » (SCPP) family [4], sharing as a common ancestor Hevin [6], and more precisely the long N-terminal domain that distinguishes it from the related protein SPARC (secreted protein, acidic and rich in cystein)/Osteonectin [7]. The SCPP share a flexible structure, and many contain numerous acidic aminoacid residues, which favor interactions with crystals (review in [8]). The SIBLINGs, more specifically, have acidic pI (with the exception of MEPE), and display in their sequence a proline-rich stretch (basic), consensus sites for casein-kinase, an arginine-glycine-aspartic acid (RGD) sequence binding intergrin family receptors, and (apart for BSP) one or several ASARM (acidic serine-aspartate rich MEPE associated) peptides, which have a high affinity for hydroxyapatite and appear to be potent regulators of mineralization [9, 10]. The SIBLINGs also display a high degree of post-translational modification (phosphorylation, sulfatation and/or glycosylation) which varies for a given protein in time (cellular differentiation) and space (tissue), and directly affects their biological functions (review in [11]). In bone, the SIBLINGs are expressed by cells of the osteoblast lineage, DMP-1 and MEPE being mostly restricted to osteocytes. OPN and BSP at least are also expressed by hypertrophic chondrocytes and osteoclasts. SIBLINGs are also present in multiple non-mineralized tissues, expecially with secretory functions (salivary

glands, kidney, [12, 13]) and by cancer cells in which they favor metastatic processes, particularly targeting bone (review in [14]).

## **Regulators of matrix mineralization and bone remodeling**

DMP1 and MEPE appear as prominent regulators of mineralization. DMP1 knockout mice display massive osteomalacia [15], at least in part indirectly, through impaired maturation of osteocytes, which is necessary to proper mineralization, and also increased levels of FGF-23 (a powerful negative regulator of phosphatemia [16]) as well as MEPE [17]. In contrast, MEPE knockout increases bone mass [18]. A major inhibitory factor of mineralisation (“Minhibin”, [19]) is the ASARM peptide (review in [9]), which is cleaved by cathepsins B and K from MEPE and DMP-1, circulates in blood and is responsible for the high levels of osteomalacia observed in hypophosphatemia [10]. The most studied of SIBLINGs, OPN (review in [20]), is a ubiquitous protein whose functions range from inflammation to lactation, and which has been called a cytokine [21]. The bones of OPN knockout mice [22] display a cell-autonomous defect of osteoclast recruitment and activity. This results in resorption defect, a higher trabecular bone mass in mutant bones, and a lack of response to challenges increasing bone loss, such as ovariectomy [23] and hindlimb unloading, a model of disuse bone loss [24]. Interacting with bone cells through both integrins (mostly  $\alpha v\beta 3$ ) and CD44, OPN thus appears directly involved in the regulation of cell adhesion and bone remodeling. Interestingly, OPN  $-/-$  bone is also hypermineralized, and lack of OPN partly compensates osteomalacia in mice with a knockout of tissue non specific alkaline phosphatase (TNALP), confirming that OPN also plays a part as a physiological inhibitor of organic matrix mineralization [25]. Considering that OPN and BSP (review in [26]) are the two major SIBLINGs expressed in bone forming osteoblasts, it is important to clarify the specificities

and redundancies in their respective functions.

## **The roles of BSP - insights from a mild phenotype knockout**

We generated BSP knockout (BSP<sup>-/-</sup>) mice through genetic recombination, and studied both their basal phenotype and their response to challenges [27]. BSP<sup>-/-</sup> mice develop and grow normally, but remain smaller than their wild-type counterparts throughout life (Figure 1a, b). Cortical bone is thinner in young mutant mice (Figure 1c) and progressively increases with age to reach wild type values. BSP<sup>-/-</sup> mice display early, mild matrix hypomineralization (~5% in adults), which also progressively normalizes in older mice. Previous *in vitro* data already showed that BSP promotes the formation of hydroxyapatite crystals, contrarily to OPN [28]. BSP is highly abundant in woven, primary bone [29] where it would play a direct part in mineralization, in association with an unrelated aggregating glycoprotein, bone acidic glycoprotein-75 [30, 31]. Indeed, primary marrow cultures from BSP<sup>-/-</sup> bones grow normal numbers of TNALP<sup>+</sup> (=osteoblast lineage) colonies but reduced numbers of mineralized nodules respective to wild type controls. The progressive recovery of mineralization degree in older BSP<sup>-/-</sup> mice also suggests a role in mineralization focused on primary bone. However, BSP<sup>-/-</sup> marrow cultures also display reduced expression of osteoblast-related genes, such as type I collagen, indicating that, beyond crystal nucleation, lack of BSP also affects mineralization through alterations of the osteoblast phenotype and matrix amount/composition. This confirms previous work with recombinant proteins, showing the importance of BSP, specifically the RGD containing portion, for osteoblast phenotypic regulation [32]. Osteoblast phenotype impairment could also explain why the bone formation rate (BFR) of secondary, trabecular bone of BSP<sup>-/-</sup> mice is very low (Figure 1d), with reduced osteoblast surfaces and increased osteoid surfaces and thickness. We challenged the

ability of mutant mice to repair bone with a cortical defect model. After drilling a hole in the femur proximal to the knee articulation, repair was followed up through histology, MRI and microtomography. The lack of BSP significantly delayed the filling up of the defect respective to wild-type controls, confirming the impairment of bone formation (data to be published). Surprisingly with such low BFR, trabecular bone volume is higher (~30%) in 4 month old BSP<sup>-/-</sup> long bones than in wild type (Figure 1c), indicating a concomitant reduction of bone resorption. Osteoclast surfaces and numbers are indeed reduced in mutant mice (Figure 1d), and impaired osteoclast differentiation from BSP<sup>-/-</sup> spleen and marrow cells was confirmed in vitro [27], in accordance with previous studies [33, 34]. We then asked whether the low turnover phenotype of mutant mice could be increased by a classical challenge. BSP<sup>-/-</sup> and wild type mice of both sexes were submitted to hindlimb unloading through tail suspension for 2 to 3 weeks. We found that, contrarily to the OPN knockout [24], mice lacking BSP lose bone under unloading, with increased BFR and osteoclast surfaces [27].

In conclusion, BSP knockout impairs body and long bone growth and bone repair, but induces a high trabecular bone mass with low bone turnover that is, nonetheless, responsive to mechanical challenges. These data clearly contrast with the phenotype of OPN knockout bone, and highlight the specificity of BSP roles in the bone context, and on a larger scale the nonredundancy of function of SIBLING family members in skeletal biology.

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**Figure 1. Bone phenotype of BSP<sup>-/-</sup> mice.** (a) Pictures of BSP knockout (<sup>-/-</sup>) and wild type (<sup>+/+</sup>) mice ; (b) whole radiography and (c) cortical 2D microtomography of isolated femurs from <sup>+/+</sup> and <sup>-/-</sup> mice ; (d) quantification of bone formation rate (BFR) and osteoclast surfaces (Oc.S/BS) in the tibial trabecular bone of <sup>+/+</sup> and <sup>-/-</sup> mice ; (e) 3D microtomographic reconstruction of trabecular bone in the metaphysis of <sup>+/+</sup> and <sup>-/-</sup> femurs. The original data were published in Malaval et al., J Exp Med 205:1145-1153 - Copyright belongs to the authors.



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